(12) UK Patent Application (19) GB (11) 2 252 323 (13) A

(43) Date of A publication 05.08.1992

(21) Application No 9202100.5

(22) Date of filing 31.01.1992

(30) Priority data
(31) 9102157 (32) 01.02.1991 (33) GB

(71) Applicant
The Secretary of State for Defence
Ministry of Defence, Whitehall, London, SW1A 2HB,
United Kingdom

(72) Inventors
Richard William Titball
Alice Mary Killeen

(74) Agent and/or Address for Service
P B Lockwood
The MOD (PE), Room 2121, Empress State Bldg,
Lillie Road, Fulham, London, SW6 1TR,
United Kingdom

(51) INT CL[5]
C12Q 1/68, C07H 21/04

(52) UK CL (Edition K)
C3H HB4
C6Y Y403
G1B BAC B104 B200 B220 B221 B223
U1S S1289 S1334 S1337

(56) Documents cited
J. Virol. Methods 1986, 13, 55–62

(58) Field of search
UK CL (Edition K) C3H HA4, G1B BAC
INT CL[5] C07H 15/04, C12N 15/11, C12Q 1/68
Online databases: AUGENES, WPI; DIALOG/BIOTECH

(54) Detection of *Herpesvirus simiae*

(57) The close relationship between *Herpesvirus simiae* (B virus), SA–8, HSV–1 and HSV–2 makes them difficult to differentiate by immunological methods; the lethal effects of the former virus in man making the development of a differential test desirable. There is provided a method for identifying B virus DNA by amplifying a characteristic double stranded oligonucleotide sequence from it using PCT techniques and oligonucleotides which can be used to prime polymerase chain reactions to amplify said polynucleotide sequence specific to said B virus. Oligonucleotides which can be labelled and used as probes capable of hybridizing specifically with the amplification products produced by positive PCRs are described enabling the differentiation of *Herpesvirus simiae* (B) virus from the most likely causes of false positives.

GB 2 252 323 A

At least one drawing originally filed was informal and the print reproduced here is taken from a later filed formal copy.

Fig.1.

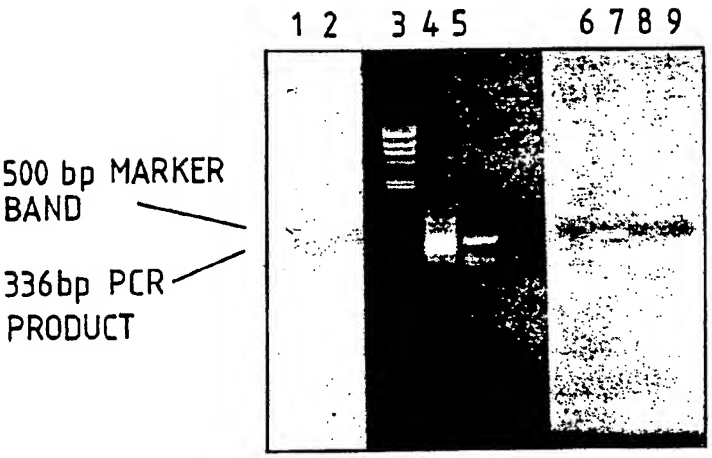


Fig.2.

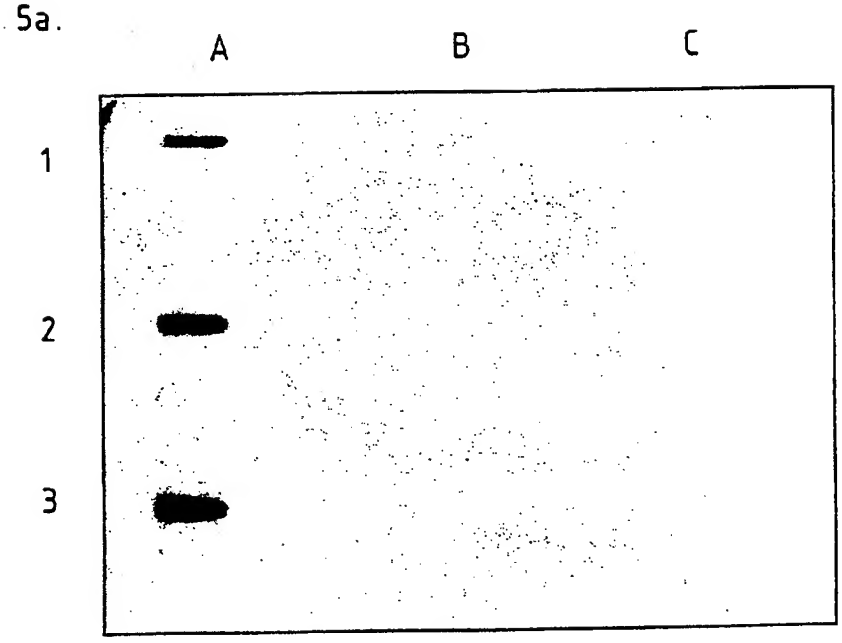
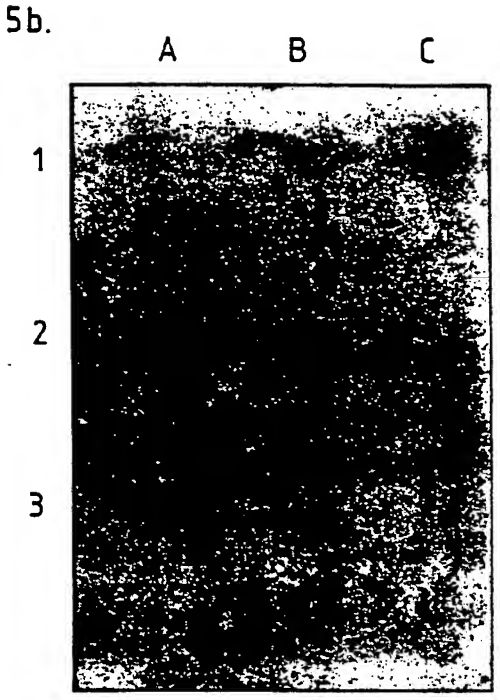


Fig.3.



VIRUS DETECTION METHOD AND OLIGONUCLEOTIDES FOR USE THEREIN.

The present invention relates to oligonucleotides having application as primers for polymerase chain reaction (PCR) amplification of a DNA sequence characteristic of Herpesvirus simiae (also known as B virus), to oligonucleotide probes for detecting said DNA sequence and to methods of detecting said virus sequence and or virus using said amplification and oligonucleotide probes.

The need to screen simian species for simian herpesvirus B is made critical by its serious effects in man, proving fatal in many cases. Prior to the handling of any such animals, whether for research or zoological purposes, it is desirable to detect the presence of this virus in order that appropriate steps might be taken to avoid human infection. The close relationship between B virus, herpes simplex virus types 1 and 2 (HSV-1, HSV-2), and SA8 virus has hindered differential identification of these viruses by immunological methods.

The present inventors have developed oligonucleotides which are capable of high specificity hybridization with B virus DNA such that a characteristic sequence, the B virus 10K gene, may be amplified by using them as primers for a polymerase chain reaction (PCR). Use of such primers to amplify the 10K gene sequence provides the opportunity to detect an increased amount of this characteristic DNA material by other means.

The specificity of PCR amplification is known to be as specific as the hybridization of the most specific primer to the target sequence. Even the relatively specific PCR primers of the invention are capable of producing amplification of DNA sequences which they hybridize to but which contain different intervening sequences to the target virus sequences. To increase the specificity of detection of the target 10K sequence the present inventors have further devised relatively small oligonucleotide probes with which they can determine with more certainty whether the product of the PCR amplification is that of the target DNA. By use of the PCR step followed by the probe hybidization step the present inventors have further provided a method for the detection of Herpesvirus simiae which, by use of preferred sequences, is capable of distinguishing the target sequence from those with over 80% homologous sequences and which thus provides a novel B virus

detection method with reduced occurence of false positives.

Thus the present inventors have sequenced a region of the B virus genome containing the B virus 10K gene and determined it to be suitably characteristic of the Simian viral strain to be useful for sample identification purposes. They have further devised polymerase chain reaction primers which are capable of initiating amplification of this sequence in a specific manner. In order to more specifically determine whether 10K gene amplification has occurred they have devised a test method whereby samples which have been treated with the primers under PCR supporting conditions are then probed using one or more labelled oligonucleotide probes specific for characteristic parts of that sequence.

The criteria used to select candidate PCR primers and oligonucleotide probes are difficult to fulfil when choosing oligonucleotides specific for B virus. The region of the B virus genome containing the 10K gene has a GC content of 67.8%, undesirably high for the initial genomic denaturation which is crucial for efficient annealing of the primers to the target template. Optimum size oligonucleotide sequences of 18 to 30 bases with desired 40 to 60% GC content are rare and 4 and 5 consecutive identical nucleotides occur frequently. These factors, coupled with the high degree of conservation among sequenced herpesvirus 10K genes (see Table 1) limit the number of candidate PCR primer pairs and oligonucleotide probes for the B virus 10K gene they have selected as the target for the present primers and probes.

When selecting PCR primers which specifically detect their target, it must be considered that for PCR amplification of DNA to occur a pair of primers must hybridize to opposite strands of the target sequence. The primers must be oriented with their 3' ends pointing towards each other; it is known that for PCR primers to be specific that specificity of the 3' end is crucial. In the correct PCR environment, an amplified DNA fragment corresponding to the distance between the primers is produced. A false positive is only seen if non-specific hybridization occurs such that a primer pair has hybridized to opposite strands of the target DNA and an 'imposter' sequence

of the correct size is amplified. Consequently, as stated above, PCR amplification specificity is dependent on that of the hybridization of the most specific primer to its target sequence.

In its broadest aspect, the present invention provides a method for the detection of Herpesvirus simiae (B virus) DNA comprising (a) carrying out a polymerase chain reaction amplification on a sample to be analysed using a pair of oligonucleotide primers of 18 or more bases each that are targeted to amplify the double stranded DNA sequence I:

Sequence I:

5' ACGCG CGTGA TTCAC GCCGA GACAC CCCCT GTGGT TTCTC CCCCT ACCCC
3' TGCGC GCACT AAGTG CGGCT CTGTG GGGGA CACCA AAGAG GGGGA TGGGG

GAGCC AACCC CTCAC GATCC CCACC CGGCA TGGAA CCCCT GCGAC TCGCC
CTCGG TTGGG GAGTG CTAGG GGTGG GCCGT ACCTT GGGGA CGCTG AGCGG

GACGC AGAGT CGCTG CTTTC GGAAA CCTCC GTGAT CCCCC TCACC CCGCC
CTGCG TCTCA GCGAC GAAAG CCTTT GGAGG CACTA GGGGG AGTGG GGCGG

GGCCC AGACC CCCGA GGCGT ACTAC ACGGA GAGCG ACGAC GAGAC GGCCG
CCGGG TCTGG GGGCT CCGCA TGATG TGCCT CTCGC TGCTG CTCTG CCGGC

CGGAT TTTCT GGTGC GGATG GGGCG GCAGC AGACG GCGAT CAGGC GTCGC
GCCTA AAAGA CCACG CCTAC CCCGC CGTCG TCTGC CGCTA GTCCG CAGCG

CGCAG GCAAA CGCGG GCCGC CGGCT TCGTG GCGGC GTTCG TCCTC GTCGC
GCGTC CGTTT GCGCC CGGCG GCCGA AGCAC CGCCG CAAGC AGGAG CAGCG

GCTGA TATCG GGTGG CCTGG GGGCC CTTAT GTGTT GGCTG GCGTA TCGCT
CGACT ATAGC CCACC GGACC CCCGG GAATA CACAA CCGAC CGCAT AGCGA

GACGA GAACG TCGCC GCCAC GAC 3'
CTGCT CTTGC AGCGG CGGTG CTG 5'

and then

(b) determining the presence and/or amount and/or nature of any reaction product of the PCR reaction and relating that to the presence and/or amount and/or nature of Herpesvirus simiae DNA initially present in the sample.

For performance of this method the present invention provides novel preferred sequence I top strand 5' end primer oligonucleotides of deoxyribonucleic acid having a base sequence according to the general sequence II:

$5'\ (M)_a$ - ACG CGC GTG ATT CAC GCC GA - $(N)_b\ 3'$ SEQUENCE II

wherein M is a base sequence of 1 to 15 bases;

N is a base sequence selected from the sequences G, GA, GAC, GAC A, GAC AC, GAC ACC, GAC ACC C, GAC ACC CC, GAC ACC CCC, GAC ACC CCC T, GAC ACC CCC TG, GAC ACC CCC TGT, GAC ACC CCC TGT G, GAC ACC CCC TGT GG and GAC ACC CCC TGT GGT;

and a and b are independently selected from 0 and 1 .

Preferably M is a base sequence selected from the sequences A, GA, AGA, C AGA, GC AGA, CGC AGA, A CGC AGA, CA CGC AGA, GCA CGC AGA, G GCA CGC AGA, CG GCA CGC AGA, CCG GCA CGC AGA, G CCG GCA CGC AGA, CG CCG GCA CGC AGA and TCG CCG GCA CGC AGA.

The present invention further provides novel sequence I 3' end primer oligonucleotides of deoxyribonucleic acid having the base sequence III:

$5'\ (X)_a$ - GTC GTG GCG GCG ACG TTC - $(Y)_b\ 3'$ SEQUENCE III

wherein X is a base sequence of 1 to 15 bases;

Y is a base sequence selected from the sequences T, TC, TCG, TCG T, TCG TC, TCG TCA, TCG TCA G, TCG TCA GC, TCG TCA GCG, TCG TCA GCG A, TCG TCA GCG AT, TCG TCA GCG ATA, TCG TCA GCG ATA C, TCG TCA GCG ATA CG and TCG TCA GCG ATA CGC.

and a and b are independently selected from 0 or 1.

Preferably a is 0, but where it is 1, X is preferably a base sequence selected from the sequences G, GG, GGG, GGG G, GGG GG, GGG GGG, GGG GGG G, GGG GGG GG, GGG GGG GGG, GGG GGG GGG T, GGG GGG GGG TG, GGG GGG GGG TGG, GGG GGG GGG TGG G, GGG GGG GGG TGG GG and GGG GGG GGG TGG GGG.

In each of the sequences above the letters A, C, G, and T relate to the bases of a deoxyribonucleic acid chain in which A represents adenine, C represents cytosine, G represents guanine and T represents thiamine or, in each case, a base having equivalent base pairing characteristics to these. For example, T also represents uracil; other such equivalents being well known to a man skilled in the art.

The present invention further provides novel probe oligonucleotides of deoxyribonucleic acid having up to 336 bases and comprising the sequence IV or its complementary base sequence:

5'- CTC TGC GTC GGC GAG TCG CA -3' SEQUENCE IV

and labelled oligonucleotide probes comprising them. The letters A, C, G and T in sequence III have the same meanings as for sequences I and II above.

Preferred sequences are the 20 base sequence IV itself, its complementary sequence (shown in Table 2 as bases 679 to 698), and any sequence consisting of these sequences flanked by sequences

complementary to the target 10K sequence which is to be detected. Thus the complementary sequence to sequence IV, if flanked, is preferably flanked by sequences having the same order as those shown flanking it in Table 2. As will be understood by the skilled man preferred flanking sequences for the sequence IV will be of the same order as those complementary to those flanking said bases 679 to 698 of Table 2. Preferred flanked sequences are up to 50 bases in total.

Flanking sequence accuracy for the probe is not as critical as for the primers as only part of the probe needs to bind to the target whereas the 3' end of a primer must be so bound. For both primer and probe use it will be realised that a variation of 1 or 2 bases in 20 in the centre of the respective sequence will be possible.

The present invention also provides a method for the production of isolated sequences comprising the Herpesvirus Simiae (B virus) 10K gene sequence, said method comprising use of a pair of oligonucleotide primers and a poly or oligo nucleotide base pair sequence comprising such 10K gene sequence as a template to carry out a polymerase chain reaction, wherein the gene 5' end primer is of general sequence II. Preferably the gene 3' end primer is of general sequence III.

The present invention further provides a method for the detection of Herpes Simiae B virus comprising (a) carrying out a polymerase chain reaction amplification on a sample to be analysed using a pair of oligonucleotide primers wherein one primer is of general sequence II, and the other primer is of general sequence III wherein the template for the reaction is provided by B virus 10K gene base pair sequence present in said sample, and (b) determining the presence and/or nature of any reaction product.

A typical method of determining whether PCR amplification has occurred is by gel chromatography eg. electrophoresis, of the sample against a known product and observation of the presence of said product under ultraviolet light. A typical sample for analysis will be a viral genomic DNA isolated from swab cultures, eg, by SDS/phenol extraction although any suspect DNA may be so analysed. Generally known PCR

methods may be used.

The present invention still further provides a method for the detection of Herpes Simiae B virus comprising:

(a) carrying out a polymerase chain reaction amplification on a sample to be analysed using a pair of oligonucleotide primers wherein one primer is of general sequence II and the other primer is of general sequence III and wherein the template for the reaction is provided by B virus 10K gene base pair sequence present in the sample and

(b) probing any oligonucleotide amplified by the step (a) under hybridization conditions with a labelled oligonucleotide comprising sequence IV or a sequence complementary thereto to determine the match between the probe and the amplification product sequences.

The results of probing may be determined by any standard method and are conveniently compared with concurrent probing of control samples of positive and negative nature, as will be known to the man skilled in the art. Eg. Positive controls may comprise one or more target viral DNA concentrations; negative controls may comprise one or more concentrations of related DNA which it is not wished to be detected. Probing with radiolabelled oligonucleotides of general sequence IV may be examined by use of standard autoradiography techniques.

It will be understood that the method of the present invention also provides a method of detecting the 10K gene sequence of B virus in DNA samples smaller than the complete viral genome. The target sequence for the probes comprising sequence IV are their complementary sequences in each case, and will be found on one or other strand of the viral DNA sequence at the 10K locus. Thus any sequence containing complementary sequence to part of the probe sequence can, in theory, have the probe hybridize to it under appropriate conditions. Methods for controlling the stringency of DNA hybridization are well known such that a set number of bases must be matched before hybridization will occur, eg. by selection of temperature. In this way the

specificity of the probe for binding to the target sequence and to non-target sequences may be controlled to give the specificity required.

Preferred sequences for the steps (a) and (b) above are those as described as preferred for sequences II and III, and IV and its complementary sequences respectively. Preferred conditions will be those only allowing hybridization at homology over that of the most specific primer or probe with false positive sequences.

Most preferably each of the oligonucleotides used in the steps (a) and (b) are of 18 to 30 bases long, although use of some longer oligonucleotides may provide high specificity if high stringency hybridization conditions are used. It is particularly preferred that no more than three consecutive identical nucleotides should be included in the sequences in order to minimize false positive homologies. Advantageously the GC content of the primers should be from 40 to 60%.

Most preferably step (a) is carried out using a pair of primers having only the 18 base sequence II (where a and b are 0) and the 20 base sequence III (where a and b are 0) respectively.

Most preferably the probing of step (b) will be carried out using a labelled oligonucleotide comprising the sequence IV above or its complementary sequence, conveniently being a labelled oligonucleotide having only the bases of sequence IV.

PREPARATION OF OLIGONUCLEOTIDES:

Preparation of the oligonucleotides of the present invention may be carried out using standard synthsizer apparatus. Typical of such apparatus is the ABI (Applied Biosystems Ltd) automated DNA synthesizer and a typical method uses beta-cyanoethyl diisopropyl phosphoramidite (CE-phosphoramidite) monomers and chemistry (Gait, M J Editor. Oligonucleotide Synthesis- a practical approach, IRL Press,

Oxford, 1984) based on a method by Beaucage and Caruthers, published in Tetrahedron Letters 22, 1859-1862 (1981), using dimethylamino -phosphoramidites. All reagents are commercially available from a number of suppliers including ABI.

In use the 3'-terminal of the sequence is attached to a silica gel and synthesis proceeds from the 3' to the 5' end. Non-isotopic markers such as biotin, chemiluminescent labels or fluorescent dyes are attached by way of application of an aminohexylphosphate Aminolinker 2 reagent (ABI part No 400808) followed by as base at the 5' terminus of the sequence to which the marker can be linked.

In the ABI system, after completion of the synthesis the silica gel, which is of controlled pore size, is treated with 35% v/v Ammonia in water at room temperature to hydrolyse off the sequence prepared. The treatment is continued at 55°C for several hours to remove protecting groups from the phosphate groups to give the active oligonucleotide. The active amino-group of the amino-linker agent is uncovered by this process thus allowing subsequent labelling. Product may be purified by Sodium acetate/ethanol precipitation directly from the ammonia solution.

Alternatively oligonucleotides may be purchased, 'custom made' by companies such as Pharmacia UK Ltd and suitably purified by column chromatography such as that using gel filtration using a NAP-10 column (Pharmacia UK Ltd).

Radiolabelling will be the usual method of choice for probing purposes and is conveniently carried out using gamma ^{32}P by the method of Feinburg and Vogelstein (1984) Anal. Biochem. 137, p266-267. Similar techniques will be exemplified below.

The oligonucleotides and method of the present invention will now be described in more detail by way of exemplification only with regard to the following non-limiting Examples with reference to the Figures and background data Tables provided.

TABLES: (Tables 1 and 2 are provided at the end of the specification).

Table 1: shows four sequenced herpesvirus 10K genes illustrating the high degree of conservation between them.

Table 2: shows the nucleotide sequence of the region of the B virus genome encoding the 10K gene. Major open reading frames (ORFs) with their corresponding initiation and termination codons are indicated. Arrows show the direction of the ORF. The positions of the PCR primers and oligonucleotide probe sequences are indicated. Also indicated are the restriction fragments from which the sequence may be determined and corresponding restriction endonuclease sites. 1. 3.3Kb Bam` HI/Bam HI fragment; 2. 0.9Kb Sst I/Sst I fragment; 3. 1.6Kb Sal I/Sal I fragment.

FIGURES:

Figure 1: shows an agarose gel analysis of B virus DNA synthesised by PCR at different annealing temperatures. Gels were 0.8% agarose. 10 microlitres of PCR reaction mixture was examined in each case. The oligonucleotides used to prime DNA synthesis were PCR-10K and PCR4-10K as referred to below.

Figures 2 and 3: show hybridization of PROBE-10K to herpesvirus DNA samples. Figure 2 is an autoradiograph of a filter after hybridization with PROBE-10K at 53°C (T_d -15°C) and Figure 3 is an autoradiograph of a filter after hybridization with PROBE-10K at 43°C (T_d -25°C); autoradiography being carried out overnight at -70°C.

HOMOLOGIES OF PREFERRED SEQUENCES:

Preferred oligonucleotides having sequences the sequences I, II and III were checked with known sequences to determine homology in order to determine potential specificity.

A preferred primer of sequence II comprising an oligonucleotide of sequence:

5'- ACG CGC GTG ATT CAC GCC GA - 3'

was designated **PCR1-10K**

A preferred primer of sequence III comprising an oligonucleotide of sequence:

5'- GTC GTG GCG GCG ACG TTC -3'

was designated **PCR4-10K**

A preferred probe of sequence IV comprising an oligonucleotide of sequence:

5'- CTC TGC GTC GGC GAG TCG CA -3'

was designated **PROBE-10K**

The sequence alignment of the 10K gene homologues of B virus (SHBV10K), HSV-1 (HSV1-10K), PRV (PRV11K) and VZV (VZVUS1) are shown in **Table 1.** Sequences were aligned using the CLUSTAL computer programme, (Higgins and Sharp, (1988) Gene 73, P237-244). Initiation (ATG) and termination codons (TAA, TAG or TGA) for each gene are underlined. The PCR primers and oligonucleotide probe target sequences are identified.

The sequence homologies between the preferred oligonucleotides and the 10K regions of B virus and three other Herpes viruses are shown in **Table 3.** below.

Table 3.

OLIGONUCLEOTIDE		B VIRUS	HSV-1	PRV	VZV
PCR-10K	Matched Bases	20	7	9	0
	% homology	100	35	45	0
PCR4-10K	Matched Bases	18	8	9	0
	% homology	100	44	50	0
PROBE-10K	Matched Bases	20	5	4	7
	% homology	100	25	20	35

It should further be noted that:

PCR1-10K has homology of over 80% with Bovine alkaline phosphatase, goat embryo alpha-globulin gene, L. donovani cation-transporting ATPase and its 1b gene and Erwinia carotovora araC gene.

PCR-10K has homology of over 80% with HSV-1 U_L, HSV-1 exonuclease gene and HSV-2 genes encoding ribonucleotide reductase (90%) and the Epstein-Barr virus (EBV) complete genome. PRV above is pseudorabies virus.

PROBE-10K has homology with EBV (81%).

PCR1-10K has 76% homology with HSV, EBV, Human Cytomegalovirus (HCMV), PRV (whole) and varicella zoster virus (VZV) (whole).

PCR4-10K has homology with HSV at 79 to 84% at 49 loci, with EBV at 76 and 84% at two loci, with HCMV at 79% at four loci and with PRV at 79 to 84% at three loci. One loci of HSV-2 ribonucleotide reductase genes has 90%.

PROBE-10K has homology of 76 to 81% with HSV, EBV and HCMV.

Overall the most specific primer of the two is PCR1-10K which shows a best match with non-B virus DNA sequences of 76% and thus the PCR amplification using this will give such specificity. By use of the two primers PCR1-10K and PCR4-10K together under conditions which allow hybridization at greater than 76% homology the 10K gene can be amplified specifically to give a 336 base pair DNA product.

For unequivocal identification of the PCR-generated product, PROBE-10K will be used as an oligonucleotide probe. Hybridization conditions preferably only allow binding at over 82% homology between the probe and the target DNA, resulting in specific detection of the B virus target sequence.

EXAMPLE 1. PCR amplification of the B virus 10K gene.

The PCR machine used was a PREM™, manufactured by LEP Scientific Ltd. Sunrise Parkway, Linford Wood, Milton Keynes, MK14 6QF UK. Synthesised primers (see preparation of oligonucleotides above) were purified by gel filtration using a NAP-10 column (Pharmacia UK Ltd) followed by ethanol precipitation. Oligonucleotides were resuspended in TE buffer (10nM Tris.HCl, pH 7.4; 1mM EDTA, pH 8.0) before use.

B virus genomic DNA was taken from a strain designated prototypic B virus and was an oral isolate from a Cynomolgus monkey (Vizozo,(1975) British Journal of Experimental Pathology 56, p485-488). B virus was grown in Vero cells and total DNA (cellular and viral) was isolated

from SDS/phenol extracted cultures according to the method of Lonsdale (1979, Lancet 1, p849-851 also Wall et al (1989) Virus Research 12, p283-296). Deoxynucleotides were purchased from Pharmacia UK Ltd, Davy Avenue, Knowlhill, Milton Keynes, MK5 8PH, UK. Taq DNA polymerase and 10 X PCR buffer were purchased from Boehringer Mannheim UK, Bell Lane, Lewes, East Sussex, BN7 1LG, UK. Light mineral oil was purchased from Sigma Chemical Co Ltd, Fancy Road, Poole, Dorset, BH17 7TG, UK.

Reagents were made up as follows:

(i) Deoxynucleotide (dNTP) mix was comprised of 10mM of each of dATP, dCTP, dGTP and dTTP in sterile redistilled water; (ii) 10 x PCR buffer comprised of 100mmol/l Tris.HCl, 15mmol/l $MgCl_2$, 500mmol/l KCl and 1mg/ml gelatine all at pH 8.3.

Protocol:

The following reagents were mixed in a thin-walled tube which closely fits into the heating block of the PCR machine.

Template DNA	x	microlitres	less than 100ng
dNTP mix	8	"	final conc 200 micromolar
10x PCR buffer	10	"	
PCR1-10K	1	"	1g/l stock; 0.2micromoles
PCR4-10K	1	"	" " "
Taq DNA polymerase	0.5	"	5 units/microlitre
Sterile redistilled water	y	"	
Total	100	"	

The reaction was covered with 70 microlitres of light mineral oil and 30 to 50 cycles of amplification were performed according to the following sequence: Ramp to 94°C; 1 minute at 94°C to denature; Ramp

to 50°C; 1 minute at 50°C to anneal; Ramp to 72°C; 1 minute at 72°C to extend. Final extension time after 30 to 50 cycles is 11 minutes at 72°C. The reaction is refrigerated at 4°C until use.

Analysis was carried out on a 10 microlitre sample using gel electrophoresis through 0.8% agarose in the presence of ethidium bromide (0.5 microgram/ml). The presence of B virus DNA in the sample is indicated by visualisation of a single PCR DNA product of 336 base pairs under ultraviolet illumination.

The results of such an agarose gel analysis is shown in Figure 1 wherein 10 microlitres of reaction mixture have been examined in each case. The oligonucleotides used to prime DNA synthesis were PCR1-10K and PCR4-10K as described above.

Lanes are shown as follows:

Lane 1: Molecular weight markers.
Lane 2: 100ng B virus DNA template; 45°C annealing temperature.
Lane 3: Molecular weight markers.
Lane 4: Miscellaneous.
Lane 5: 100ng B virus DNA template; 50°C annealing temperature.
Lane 6: Molecular weight markers.
Lane 7: 100ng B virus DNA template: 60°C annealing temperature.
Lane 8: 100ng B virus DNA template: 61°C annealing temperature.
Lane 9: 100ng B virus DNA template: 62°C annealing temperature.

Factors affecting amplification and specificity.

As the activity of Taq polymerase decreases during cycling, an additional 0.5 microlitres may be added to the reaction mixture after about 30 cycles if the reaction is to be continued.

It is preferred to ensure that the samples maintain each incubation temperature for the time stipulated; the heating block temperature is usually different to the temperature of the sample and use of a thermocouple to measure the temperature of a control sample is advised.

Addition of 5 to 15% of glycerol may improve amplification (see Smith et al, (1990) Amplifications, page 16 - Perkin-Elmer Corporation Publication).

The temperatures of the denaturation and extension steps above are optimal; the specificity of the annealing step may be altered freely to change the specificity of the PCR. As demonstrated in Figure 1 the maximum annealing temperature has been found to be 60°C when 100ng prototypic strain B virus is used as the target template. A single PCR product of 336 base pairs is seen at temperatures between 50 and 60°C. At 45°C, non-specific hybridization of one or both primers generated multiple PCR products. The exact choice of temperature will depend on whether problems of non-specific hybridization are encountered with the samples to be tested.

Initially, an annealing temperature of 50°C is preferred to be used and the temperature is then preferably raised to between 50 and 60°C if necessary.

EXAMPLE 2. Detection of the B virus 10K gene by oligo-probing.

Oligo-probing was carried out using the PROBE-10K probe using the following protocols:

a. Slot blotting:

Slot-blots of the DNA to be probed was prepared using 5ng, 10ng and 15ng of PCR product. Positive and negative controls were included; B virus genomic DNA controls were used at 500ng level.

The Bio-dot^{R} apparatus and Zeta-probe^{R} blotting membranes available from Bio-Rad, 32nd and Griffith Avenue, Richmond, CA 94804, are particularly suitable for such operation.

b. 5' end-labelling of oligonucleotides:

Machine synthesised PROBE-10K was purified on a NAP-10 column and then subjected to ethanol precipitation. The purified oligonucleotide was resuspended in TE buffer for use.

50ng oligonucleotide (5-10pmol), 50microCuries of gamma ^{32}P ATP (10 microCuries/microlitre), 2 microlitres x10 PNK buffer and 10 units of polynucleotide kinase (10 units/microlitre) were made up to 20 microlitres with water; the enzyme being added last. The solution was incubated for 2 to 3 hours at 37°C and then the unincorporated label was removed using a NENSORB 20 column (NEN Research Products, DuPont) following the manufacturers instructions.

NB: x10 PNK buffer content is 0.7M Tris.Cl pH 7.6; 0.1M $MgCl_2$; 50mM dithiothreitol (prepared fresh in H_2O) and 1mM spermidine (optional).

c. Southern Hybridization:

The temperature at which hybridization is conducted depends on the type of probe used and it's nucleotide content. For oligonucleotide probes the degree of mismatch between the probe and target DNA is calculated using the equation:

$$T_d = 2(A + T) + 4(G + C)\ °C$$

where T_d is the melting point of the DNA duplex. A, T, G and C are the number of bases adenine, thiamine, guanine and cytosine, respectively, in the probe sequence. A mismatch of one base in the DNA duplex is tolerated for each 5°C of hybridization temperature below T_d.

For PROBE-10K, T_d was found to be 68°C. The procedures below were carried out at a temperature of T_d -15°C (53°C). Theoretically this should allow 15% mismatch between probe and target DNA (ie: a 3 base mismatch). In practice the conditions are more stringent than this (see Results), so using T_d -15°C should ensure specific detection of the B virus 10K gene while allowing for possible variation in the target sequence during evolution of different strains of B virus. If the probe hybridizes to non-B virus control DNA the hybridization temperature should be raised by 5°C. If the probe fails to recognise B virus DNA due to strain variation, the temperature should be decreased by 5°C intervals until a satisfactory result is obtained with the controls.

d. Pre-hybridization:

The membranes were placed in a petri-dish and covered with an excess (about 20ml) of pre-hybridization solution containing 52.59 g/l NaCl; 26.46 g/l Na citrate and 3.12 g/l NaH_2PO_4 with 5 g/l of blocking agent (purchased from Boehringer Corporation Ltd); at pH 7.0 and incubated for 2 hours at the chosen temperature.

e. Hybridization:

The pre-hybridization solution was poured off and replaced with fresh pre-hybridization solution at 0.1 to 0.3 ml/cm^3 of membrane. The labelled denatured probe was added using approximately 0.5 pmol of labelled oligonucleotide per ml of hybridization solution and the petri-dish lid was sealed with plastic tape and placed in a heat-sealed plastic bag. Incubation at the hybridization temperature selected was carried out overnight; very gentle agitation was applied using a rotating table in some cases.

f. Post-hybridization:

The petri-dishes were carefully unsealed and the hybridization solution was disposed of (according to local safety regulations). The membranes were transferred into a sandwich box containing 300ml wash solution (6 x SSC, 0.1% sodium dodecyl sulphate (SDS). After washing twice for 15 minutes each at the appropriate temperature with gentle agitation the wash solution was discarded and washing was carried out using 300ml of stringent wash solution (2 x SSC, 0.1% SDS) for 30 minutes at the appropriate temperature and repeated if necessary. The membranes were laid onto a piece of blotting paper to air dry for 30 minutes.

g. Autoradiography of membranes:

The radiography was carried out at -70°C. The filters were exposed with MP-Hyperfilm purchased from Amersham International PLC, the filters and MP-Hyperfilm being placed in a Kodak X-Omatic cassette filled with Kodak X-Omatic regular intensifying screens. The cassettes were incubated at -70°C for at least five hours before developing the film.

h. Re-use of blotting membranes:

Blotting membranes can be re-probed if the old probe is stripped off. The filter is incubated at 45°C for 30 minutes in 100ml of 0.1 x SSC, 0.1% SDS, 0.2M Tris.HCl at pH 7.5. Autoradiography is performed to check that all the old probe has been removed and cleared filters are then ready for pre-washing or may be stored at 4°C until required.

Results:

Slot blots were prepared using samples of PCR-amplified B-virus 10K gene and genomic DNA from B virus (prototypic strain, Vizozo, 1975 above), HSV-1 (Strain Glasgow Syn 17$^+$, Brown et al (1973), Journal of General Virology, 18 p329-346) and PRV (Davidson and Wilkie, (1983) Journal of General Virology, (1983), 64 p1927-1942.). DNA from bacteriophage M13 was used as a negative control. Results are shown in Figures 2 and 3.

The blots were probed at T_d -5°C, T_d -15°C and T_d -25°C.

At T_d -5°C (63°C) no hybridization occurred. The probe was 100% homologous to the B virus genomic DNA and PCR product. This result indicates that the conditions described, which empiracally should allow binding at 95% homology between probe and target, are in practice more stringent.

At T_d -15°C (53°C; Figure 2) the probe hybridized to the B virus DNA samples (PCR product and genomic DNA) but not to the HSV-1 or PRV DNA.

At T_d -25°C (43°C; Figure 3) the probe hybridized to the B virus DNA samples (PCR product and genomic DNA) but not to the HSV-1 or PRV DNA.

The probe showed a high specificity for B virus DNA under conditions examined. PROBE-10K remained specific for B virus even when 25% mismatch between probe and target was theoretically possible (T_d -25°C)

The upper temperature limit for the hybridization thus lies somewhere between 53 and 63°C, however, for initial use in screening a hybridization temperature of 53°C (T_d -15°C) should allow specific binding of the B virus target while accommodating possible DNA sequence variation among B virus isolates.

The precise concentrations of virus PCR product and DS M13 DNA control applied to the Zeta-probe^{R} filter for Figures 2 and 3 are shown below.

Row and column details for the filter of FIGURE 2: 53°C (T_d -15°C)

Column / Row	A	B	C
1	5ng B virus PCR product	DS M13 DNA	500ng B virus genomic DNA
2	10ng B virus PCR product		500ng HSV-1 genomic DNA
3	15ng B virus PCR product		500ng PRV genomic DNA

Row and column details for the filter of FIGURE 3: 43°C (T_d -25°C)

Column / Row	A	B	C
1	5ng B virus PCR product	500ng B virus genomic DNA	DS M13 DNA
2	10ng B virus PCR product	500ng HSV-1 genomic DNA	
3	15ng B virus PCR product	500ng PRV genomic DNA	

TABLE 1.

```
Gap penalty =    4 Cut-off =  10
Variable gap penalty =    F   Window =  10   Filtering =  2.5
 *  :-> Exact match across all seqs.;      .  :-> Conservative matches across all.
                                                                    PCR1-10K
SHBV10K          :  CGGCGCATAAATGCCCCCCGCCCCGCTCGCCGGCACGCAGAACGCGCGTGATTCACGCCG
HSV1-10K         :  ----------GGCGCATAAAGGGCCGGTGGTCCGCCTAGCCGCAGCAAATTAAAAATCGTG
PRV11K           :  --------------------------TTAAATACCGGGAGAACCGGCCCGCCCGCATTCCG
VZVUS1           :  ------------------------------------------------------------

                    ACACACCCCCTGTGGTTTCTCCCCCTACCCCGAGCCAACCCCTCACGATCCCCACCCGGC
SHBV10K          :  AGTCACAGCGACCGCAACTTCCC---ACCCGGAGCTTTCTT----------CCGGCCTCG
HSV1-10K         :  ACATGCCCACCGCCGCCCCCGCCGACATGGACACGTTCGACCCCAGCGCCCCCGTCCCGA
PRV11K           :  ------------------------ATGGCCGGACAAAACACCATGGAGGGTGAGGCCG--
VZVUS1           :                                 *                    **
                                      PROBE-10K
                    ATGGAACCCCTGCGACTCGCCGACGCAGAGTCGCTGCTTTCGGAAACCTCCGT---GATC
SHBV10K          :  ATGACGTCCCGGCTCTCCGATCCCAACTCCTCAGCGCGATCCGACATGTCCGTGCCGCTT
HSV1-10K         :  CGAGCGTCTCTAACCCGGCCGCCGACGTCCTGCTGGCCCCCAAGGGACCCCGCTCCCCGC
PRV11K           :  -TGGCCTTACTGATGGAAGCGGTGGTAACCCCGCGAGCGCAACCAAATAATACAACGATA
VZVUS1           :        *
                    CCCCTCACCCCGCCGGCCCAGACCCCCG---AGGCGTACTACACGGAGAGCGACGACGAG
SHBV10K          :  TATCCCACGGCCTCGCCAGTTTCGGTCG---AAGCCTACTACTCGGAAAGCGAAGACGAG
HSV1-10K         :  TGCGCCCCCAGGACGACTCG-------G---ACTGCTACTACAGCGAGAGCGACAACGAG
PRV11K           :  ACAGCCATACAACCGAGCCGTTCGGCTGAAAAGTGTTATTATAGTGATAGCGAAAATGAA
VZVUS1           :     *       **           *   *    ** **    ** *****  * **

                    ACGGCCGCGGATTTTCTGGTGCGGATGGGGCGGCAGCAGACGGCGATCAGGCGTCG----
SHBV10K          :  GCGGCCAACGACTTCCTCGTACGCATGGGCCGCCAACAGTCGGTATTAAGGCGTCG----
HSV1-10K         :  ACGCCCAGCGAGTTCCTGCGCCGCGTGGGACGCCGGCAGGCGGCGCGCCGGAGACG----
PRV11K           :  ACGGCAGATGAATTTTTGCGTCGAATTGGAAAATATCAACATAAAATATATCATCGTAAA
VZVUS1           :   ** *    ** **  *  . **  * **       **              **

                    -----CCGCAGGCAAACGCGGGCCGCCGGCTTCGTGGCGGCGTTCGTCCTCGTCGCGCTG
SHBV10K          :  -----ACGCAGACGCACCCGCTGCGTCGGCATGGTGATCGCCTGTCTCCTCGTGGCCGTT
HSV1-10K         :  -----CCGCCGCTGCCTGATGGGCGTCGCGATCAGCGCCGCCGCGCTGGTCATCTGCTCG
PRV11K           :  AAATTCTGTTATATTACGCTAATAATTGTCTTTGTATTTGCTATGACGGGAGCGGCCTTT
VZVUS1           :       *                 *   *       **
                                                                  PCR4-10K
                    ATATCGGGTGGCCTGGGGGCCCTTATGTGTTGGCTGGCGTATCGCTGACGAGAACGTCGC
SHBV10K          :  CTGTCGGGCGGATTTGGGGCGCTCCTGATGTGGCTGCTCCG--------CTAAAAGACCGC
HSV1-10K         :  CTGTCGGCGCTGATCGGGGGCATCATCGCCCGGCACGTGTAGCGAGCGGGTGGTGGCCGC
PRV11K           :  GCCTTGGGATATATAACGTCACAATT--------TGTTGGATAA----------------
VZVUS1           :     * **      *   *       *
```

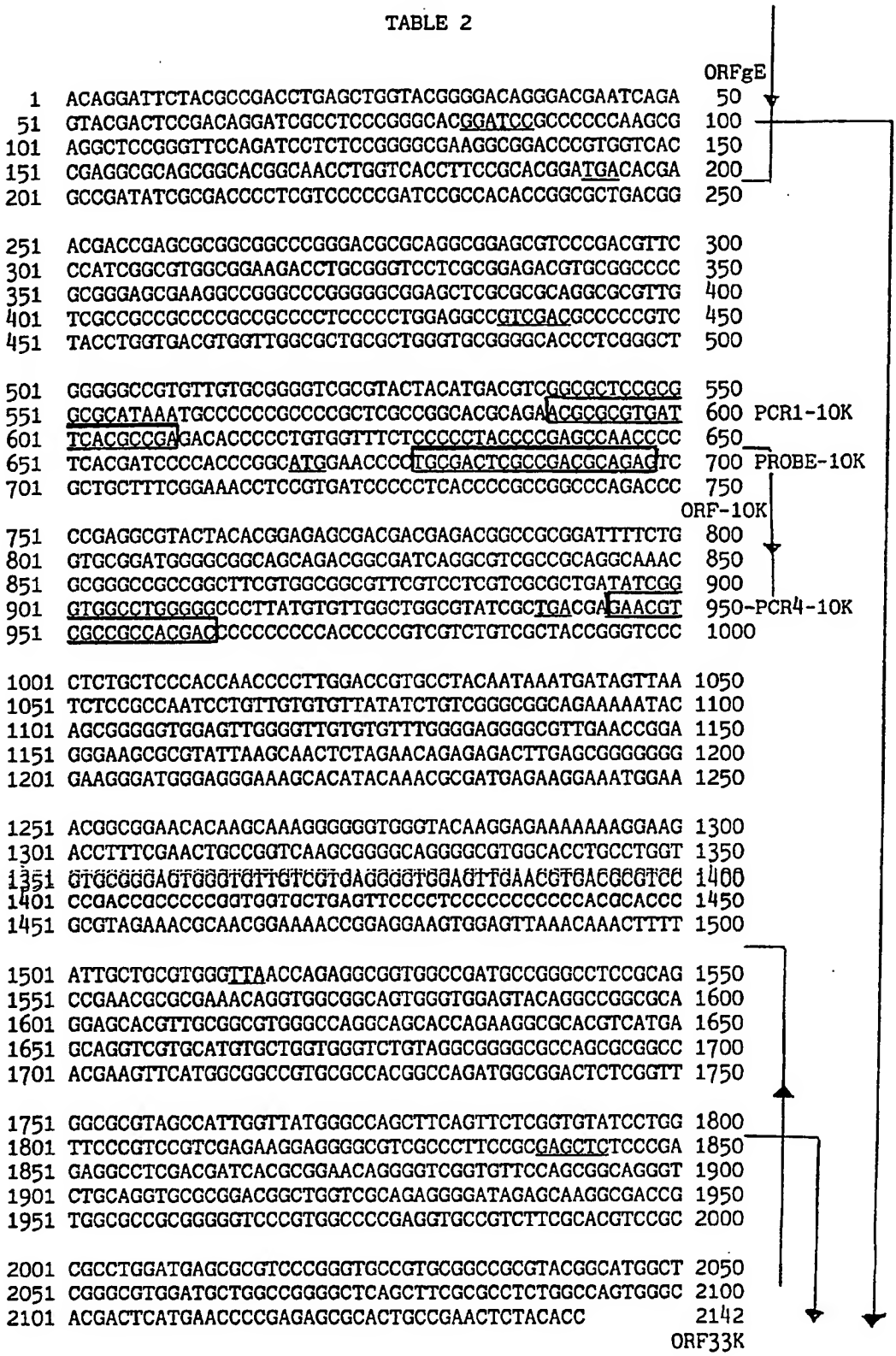
TABLE 1 contd.

```
SHBV10K     :  CGCCACGACCCCCCCCCCACCCCCGTCGTCTGTCGCTACCGGGTCCCCTCTGCTCCCACC
HSV1-10K    :  ATCGACACGCGCGTCCTTCTTGTCGTCTCTCTTCCCCCCCATCACCCCGCAATTTGCACC
PRV11K      :  CCGCCCCGCCGCGCCCAGGAG---GGGGGGTCCGGGGGGGGCGAAGCGGGCGGAGGAGAGC
VZVUS1      :  ------------------------------------------------------------

SHBV10K     :  AACCCCTTGG--------ACCGTGCCTACAATAAATGATAGTTAATCTCCGCCAATCCTGT
HSV1-10K    :  CAGCCTTTAACTACATTAAATTGGGTTCGATTGGCAATGTTGTCTCCCGGTTGATTTTTG
PRV11K      :  GAGCCACGTGGTTGTGGGCTCGGACTTGTCACAATAAA----------------------
VZVUS1      :  ------------------------------------------------------------

SHBV10K     :  TGTGTGTTATATCTGTCGGGCGG-------------------
HSV1-10K    :  GGTGGGTGGGGAGTGGGTGGGTGGGGAGTGGGTGGGTGGGGA
PRV11K      :  ---------------------------------------
VZVUS1      :  ---------------------------------------
```

TABLE 2


ORFgE
1 ACAGGATTCTACGCCGACCTGAGCTGGTACGGGGACAGGGACGAATCAGA 50
51 GTACGACTCCGACAGGATCGCCTCCCGGGGCACGGATCCGCCCCCCAAGCG 100
101 AGGCTCCGGGTTCCAGATCCTCTCCGGGGCGAAGGCGGACCCGTGGTCAC 150
151 CGAGGCGCAGCGGCACGGCAACCTGGTCACCTTCCGCACGGATGACACGA 200
201 GCCGATATCGCGACCCCTCGTCCCCCGATCCGCCACACCGGCGCTGACGG 250
251 ACGACCGAGCGCGGCGGCCCGGGACGCGCAGGCGGAGCGTCCCGACGTTC 300
301 CCATCGGCGTGGCGGAAGACCTGCGGGTCCTCGCGGAGACGTGCGGCCCC 350
351 GCGGGAGCGAAGGCCGGGCCCGGGGGCGGAGCTCGCGCGCAGGCGCGTTG 400
401 TCGCCGCCGCCCCGCCGCCCCTCCCCCTGGAGGCCGTCGACGCCCCCGTC 450
451 TACCTGGTGACGTGGTTGGCGCTGCGCTGGGTGCGGGGCACCCTCGGGCT 500
501 GGGGGCCGTGTTGTGCGGGGTCGCGTACTACATGACGTCGGCGCTCCGCG 550
551 GCGCATAAATGCCCCCCGCCCCGCTCGCCGGCACGCAGAACGCGCGTGAT 600 PCR1-10K
601 TCACGCCGAGACACCCCCTGTGGTTTCTCCCCCTACCCCGAGCCAACCCC 650
651 TCACGATCCCCACCCGGCATGGAACCCCTGCGACTCGCCGACGCAGAGTC 700 PROBE-10K
701 GCTGCTTTCGGAAACCTCCGTGATCCCCCTCACCCCGCCGGCCCAGACCC 750
ORF-10K
751 CCGAGGCGTACTACACGGAGAGCGACGACGAGACGGCCGCGGATTTTCTG 800
801 GTGCGGATGGGGCGGCAGCAGACGGCGATCAGGCGTCGCCGCAGGCAAAC 850
851 GCGGGCCGCCGGCTTCGTGGCGGCGTTCGTCCTCGTCGCGCTGATATCGG 900
901 GTGGCCTGGGGGCCCTTATGTGTTGGCTGGCGTATCGCTGACGAGAACGT 950-PCR4-10K
951 CGCCGCCACGACCCCCCCCCCACCCCCGTCGTCTGTCGCTACCGGGTCCC 1000
1001 CTCTGCTCCCACCAACCCCTTGGACCGTGCCTACAATAAATGATAGTTAA 1050
1051 TCTCCGCCAATCCTGTTGTGTGTTATATCTGTCGGGCGGCAGAAAAATAC 1100
1101 AGCGGGGGTGGAGTTGGGGTTGTGTGTTTGGGGAGGGGCGTTGAACCGGA 1150
1151 GGGAAGCGCGTATTAAGCAACTCTAGAACAGAGAGACTTGAGCGGGGGGG 1200
1201 GAAGGGATGGGAGGGAAAGCACATACAAACGCGATGAGAAGGAAATGGAA 1250
1251 ACGGCGGAACACAAGCAAAGGGGGGGTGGGTACAAGGAGAAAAAAAGGAAG 1300
1301 ACCTTTCGAACTGCCGGTCAAGCGGGGCAGGGGCGTGGCACCTGCCTGGT 1350
1351 GTGCGGGAGTGGGTGTTGTCGTCAGGGGTGGAGTTGAACGTGACGCGTCC 1400
1401 CCGACCGCCCCCGGTGGTGCTGAGTTCCCCTCCCCCCCCCCCACGCACCC 1450
1451 GCGTAGAAACGCAACGGAAAACCGGAGGAAGTGGAGTTAAACAAACTTTT 1500
1501 ATTGCTGCGTGGGTTAACCAGAGGCGGTGGCCGATGCCGGGCCTCCGCAG 1550
1551 CCGAACGCGCGAAACAGGTGGCGGCAGTGGGTGGAGTACAGGCCGGCGCA 1600
1601 GGAGCACGTTGCGGCGTGGGCCAGGCAGCACCAGAAGGCGCACGTCATGA 1650
1651 GCAGGTCGTGCATGTGCTGGTGGGTCTGTAGGCGGGGCGCCAGCGCGGCC 1700
1701 ACGAAGTTCATGGCGGCCGTGCGCCACGGCCAGATGGCGGACTCTCGGTT 1750
1751 GGCGCGTAGCCATTGGTTATGGGCCAGCTTCAGTTCTCGGTGTATCCTGG 1800
1801 TTCCCGTCCGTCGAGAAGGAGGGGCGTCGCCCTTCCGCGAGCTCTCCCGA 1850
1851 GAGGCCTCGACGATCACGCGGAACAGGGGTCGGTGTTCCAGCGGCAGGGT 1900
1901 CTGCAGGTGCGCGGACGGCTGGTCGCAGAGGGGATAGAGCAAGGCGACCG 1950
1951 TGGCGCCGCGGGGGTCCCGTGGCCCCGAGGTGCCGTCTTCGCACGTCCGC 2000
2001 CGCCTGGATGAGCGCGTCCCGGGTGCCGTGCGGCCGCGTACGGCATGGCT 2050
2051 CGGGCGTGGATGCTGGCCGGGGCTCAGCTTCGCGCCTCTGGCCAGTGGGC 2100
2101 ACGACTCATGAACCCCGAGAGCGCACTGCCGAACTCTACACC 2142
ORF33K

CLAIMS

1. A method for the detection of Herpesvirus simiae (B virus) DNA comprising (a) carrying out a polymerase chain reaction amplification on a sample to be analysed using a pair of oligonucleotide primers targeted to amplify the double stranded DNA sequence I:

Sequence I:

5' ACGCG CGTGA TTCAC GCCGA GACAC CCCCT GTGGT TTCTC CCCCT ACCCC
3' TGCGC GCACT AAGTG CGGCT CTGTG GGGGA CACCA AAGAG GGGGA TGGGG

GAGCC AACCC CTCAC GATCC CCACC CGGCA TGGAA CCCCT GCGAC TCGCC
CTCGG TTGGG GAGTG CTAGG GGTGG GCCGT ACCTT GGGGA CGCTG AGCGG

GACGC AGAGT CGCTG CTTTC GGAAA CCTCC GTGAT CCCCC TCACC CCGCC
CTGCG TCTCA GCGAC GAAAG CCTTT GGAGG CACTA GGGGG AGTGG GGCGG

GGCCC AGACC CCCGA GGCGT ACTAC ACGGA GAGCG ACGAC GAGAC GGCCG
CCGGG TCTGG GGGCT CCGCA TGATG TGCCT CTCGC TGCTG CTCTG CCGGC

CGGAT TTTCT GGTGC GGATG GGGCG GCAGC AGACG GCGAT CAGGC GTCGC
GCCTA AAAGA CCACG CCTAC CCCGC CGTCG TCTGC CGCTA GTCCG CAGCG

CGCAG GCAAA CGCGG GCCGC CGGCT TCGTG GCGGC GTTCG TCCTC GTCGC
GCGTC CGTTT GCGCC CGGCG GCCGA AGCAC CGCCG CAAGC AGGAG CAGCG

GCTGA TATCG GGTGG CCTGG GGGCC CTTAT GTGTT GGCTG GCGTA TCGCT
CGACT ATAGC CCACC GGACC CCCGG GAATA CACAA CCGAC CGCAT AGCGA

GACGA GAACG TCGCC GCCAC GAC 3'
CTGCT CTTGC AGCGG CGGTG CTG 5'

and then

(b) determining the presence and/or amount and/or nature of any reaction product of the PCR reaction and relating that to the presence and/or amount and/or nature of Herpesvirus simiae DNA initially present in the sample.

2. A method as claimed in Claim 1 wherein the upper strand 5' end primer used in step (a) is of general sequence II:

5' $(M)_a$ - ACG CGC GTG ATT CAC GCC GA - $(N)_b$ 3' SEQUENCE II

wherein M is a base sequence of 1 to 15 bases,

N is a base sequence selected from the sequences G, GA, GAC, GAC A, GAC AC, GAC ACC, GAC ACC C, GAC ACC CC, GAC ACC CCC, GAC ACC CCC T, GAC ACC CCC TG, GAC ACC CCC TGT, GAC ACC CCC TGT G, GAC ACC CCC TGT GG and GAC ACC CCC TGT GGT;

and a and b are independently selected from 0 and 1 .

3. A method as claimed in Claim 1 or in Claim 2 wherein the upper strand 3' end primer used in step (a) is of general sequence III

5' $(X)_a$ - GTC GTG GCG GCG ACG TTC - $(Y)_b$ 3' SEQUENCE III

wherein X is a base sequence of 1 to 15 bases;

Y is a base sequence selected from the sequences T, TC, TCG, TCG T, TCG TC, TCG TCA, TCG TCA G, TCG TCA GC, TCG TCA GCG, TCG TCA GCG A, TCG TCA GCG AT, TCG TCA GCG ATA, TCG TCA GCG ATA C, TCG TCA GCG ATA CG and TCG TCA GCG ATA CGC.

and a and b are independently selected from 0 or 1.

4. A method for the detection of Herpesvirus simiae (B virus) DNA as claimed in any one of Claims 1 to 3 wherein step (b) is carried out by comparing the result of gel electrophoresis of the polymerase chain reaction treated sample with that of a sample of oligonucleotide of sequence I.

5. A method for the detection of Herpesvirus simiae (B virus) DNA as claimed in any one of Claims 1 to 3 wherein step (b) is carried out by probing any product oligonucleotide amplified by the step (a) under hybridization conditions with a labelled oligonucleotide of sequence IV:

5'- CTC TGC GTC GGC GAG TCG CA -3' SEQUENCE IV

or of a sequence complementary thereto, to determine the match between the probe and the amplification product sequences.

6. A method as claimed in any one of Claims 1 to 5 wherein the upper strand 5' end primer has the base sequence:

5'- ACG CGC GTG ATT CAC GCC GA -3'

7. A method as claimed in any one of Claims 1 to 6 wherein the upper strand 3' end primer has the base sequence:

5'- GTC GTG GCG GCG ACG TTC -3'

8. A test kit for carrying out a method as claimed in Claim 1 or Claim 2 comprising an oligonucleotide primer of general sequence II:

5' $(M)_a$ - ACG CGC GTG ATT CAC GCC GA - $(N)_b$ 3' SEQUENCE II

wherein M is a base sequence of 1 to 15 bases,

N is a base sequence selected from the sequences G, GA, GAC, GAC A, GAC AC, GAC ACC, GAC ACC C, GAC ACC CC, GAC ACC CCC, GAC ACC CCC T, GAC ACC CCC TG, GAC ACC CCC TGT, GAC ACC CCC TGT G, GAC ACC CCC TGT GG and GAC ACC CCC TGT GGT;

and a and b are independently selected from 0 and 1 .

9. A test kit for carrying out a method as claimed in Claim 1, 2 or 3 comprising an oligonucleotide primer of general sequence III:

5' $(X)_a$ - GTC GTG GCG GCG ACG TTC - $(Y)_b$ 3' SEQUENCE III

wherein X is a base sequence of 1 to 15 bases;

Y is a base sequence selected from the sequences T, TC, TCG, TCG T, TCG TC, TCG TCA, TCG TCA G, TCG TCA GC, TCG TCA GCG, TCG TCA GCG A, TCG TCA GCG AT, TCG TCA GCG ATA, TCG TCA GCG ATA C, TCG TCA GCG ATA CG and TCG TCA GCG ATA CGC.

and a and b are independently selected from 0 or 1.

10. A test kit as claimed in either one of Claims 8 or 9 further comprising a labelled oligonucleotide probe of up to 338 bases which comprises the sequence IV or its complementary base sequence:

5'- CTC TGC GTC GGC GAG TCG CA -3' SEQUENCE IV

11. A method for the production of isolated oligonucleotide sequences comprising the Herpesvirus Simiae (B virus) 10K gene sequence, said method comprising use of a pair of oligonucleotide primers and a poly or oligo nucleotide base pair sequence comprising such 10K gene sequence as a template to carry out a polymerase chain reaction, wherein the gene 5' end primer is of general sequence II.

12. A method as claimed in Claim 11 wherein the gene 3' end primer is of general sequence III.

13. A method for the detection of Herpesvirus simiae (B virus) comprising:

(a) carrying out a polymerase chain reaction amplification on a sample to be analysed using a pair of oligonucleotide primers wherein one primer is of general sequence II and the other primer is of general sequence III and wherein the template for the reaction is provided by B virus 10K gene base pair sequence present in the sample and

(b) probing any oligonucleotide amplified by the step (a) under hybridization conditions with a labelled oligonucleotide of up to 336 bases length comprising sequence IV or a sequence complementary thereto to determine the match between the probe and the amplification product sequences.

14. A method as claimed in Claim 13 wherein the sample to be analysed comprises a viral genomic DNA isolated from a swab culture.

15. A method as claimed in Claim 14 wherein the sample to be analysed is derived from said culture by SDS/phenol extraction.

16. A method as claimed in any one of Claims 1 to 7 and 11 to 15 wherein the polymerase chain reaction and probing are carried out under conditions substantially as described in Examples 1 and 2.

17. A test kit as claimed in any one of Claims 8 to 10 further comprising one or more of the reagents necessary for carrying out a polymerase chain reaction and/or hybridization probing.

18. A test kit as claimed in Claim 17 wherein said reagents are one or more of those described in Examples 1 and 2.

Patents Act 1977
Examiner's report to the Comptroller under Section 17 (The Search Report)

Application number

9202100.5

Relevant Technical fields

(i) UK Cl (Edition K) C3H (HA4); G1B (BAC)

(ii) Int Cl (Edition 5) C12Q 1/68; C07H 15/04; C12N 15/11

Search Examiner

C SHERRINGTON

Databases (see over)

(i) UK Patent Office

(ii) ONLINE DATABASES: AUGENES, WPI, DIALOG/ BIOTECH

Date of Search

23 MARCH 1992

Documents considered relevant following a search in respect of claims 1-18

Category (see over)	Identity of document and relevant passages	Relevant to claim(s)
A	J. VIROL METHODS 1986, 13, 55-62 Rapid Identification of Herpesvirus Simiae (B virus) DNA from clinical	1

lme - c:\wp51\doc99\fil000557

Category	Identity of document and relevant passages	Relevant to claim(s)

Categories of documents

X: Document indicating lack of novelty or of inventive step.

Y: Document indicating lack of inventive step if combined with one or more other documents of the same category.

A: Document indicating technological background and/or state of the art.

P: Document published on or after the declared priority date but before the filing date of the present application.

E: Patent document published on or after, but with priority date earlier than, the filing date of the present application.

&: Member of the same patent family, corresponding document.

Databases: The UK Patent Office database comprises classified collections of GB, EP, WO and US patent specifications as outlined periodically in the Official Journal (Patents). The on-line databases considered for search are also listed periodically in the Official Journal (Patents).

Published 1992 at The Patent Office, Concept House, Cardiff Road, Newport, Gwent NP9 1RH. Further copies may be obtained from Sales Branch, Unit 6, Nine Mile Point, Cwmfelinfach, Cross Keys, Newport, NP1 7HZ. Printed by Multiplex techniques ltd, St Mary Cray, Kent.